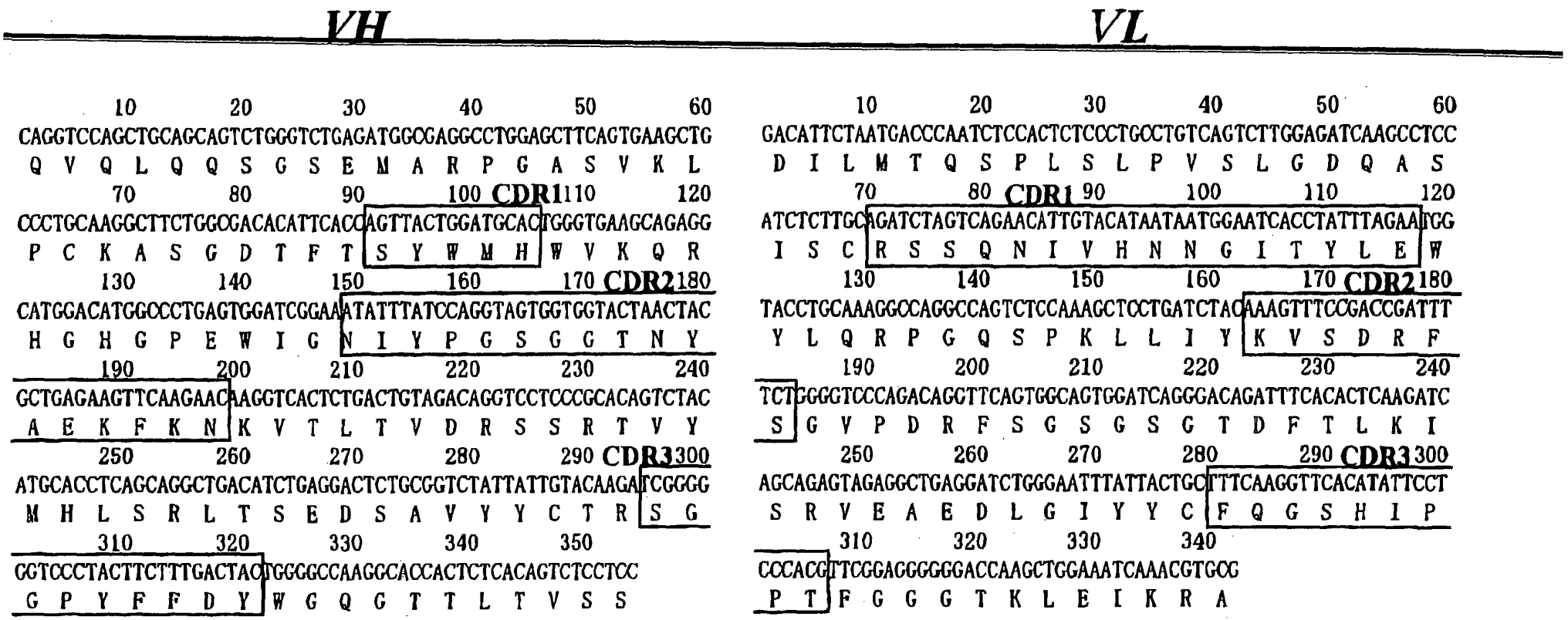


FIG. 1

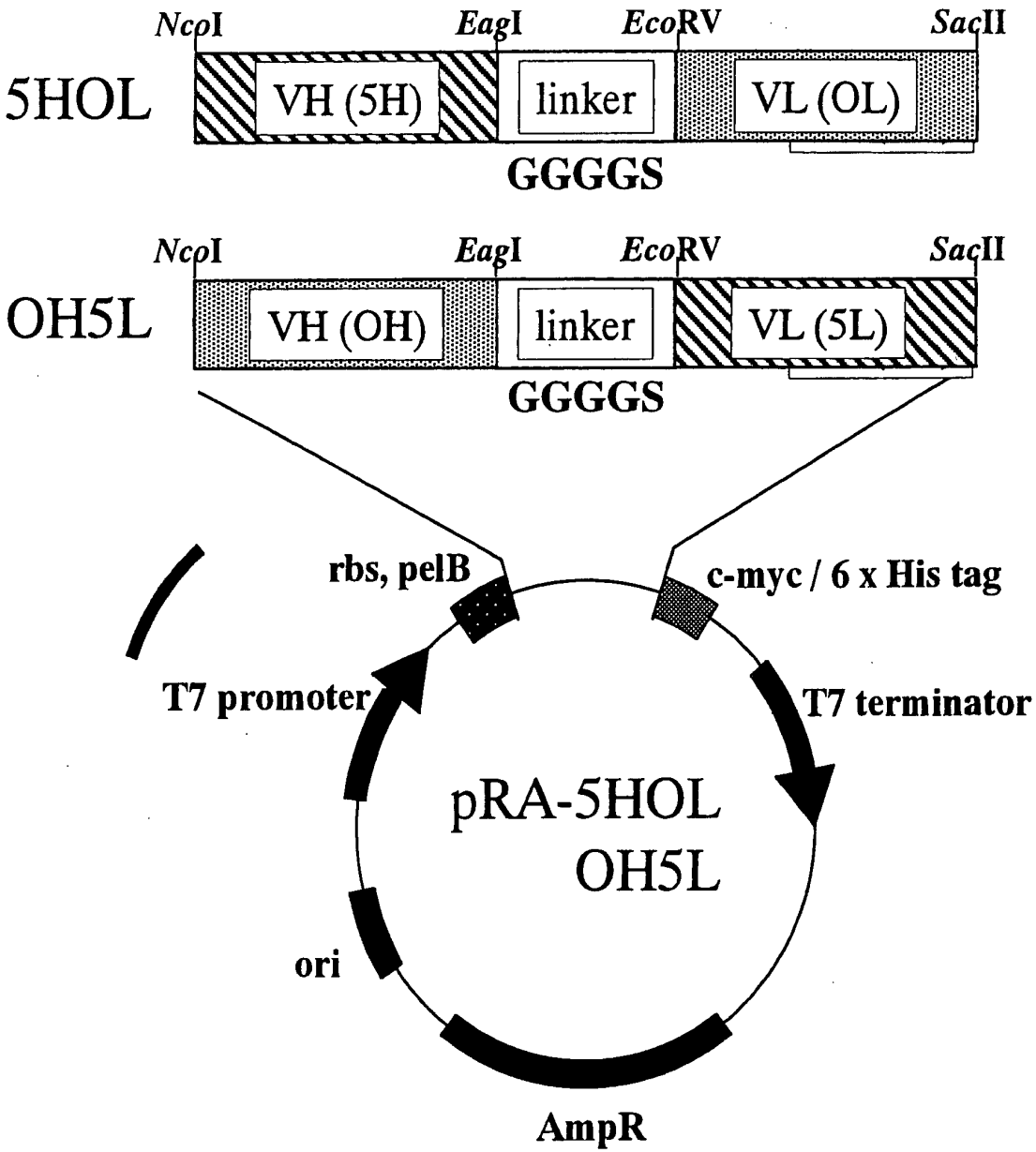

NcoI
EagI
EcoRV
SacII
5HOL
VH (5H)
linker
VL (OL)
GGGGS
NcoI
EagI
EcoRV
SacII
OH5L
VH (OH)
linker
VL (5L)
GGGGS
rbs, pelB
c-myc / 6 x His tag
T7 promoter
T7 terminator
pRA-5HOL
OH5L
ori
AmpR

FIG. 2

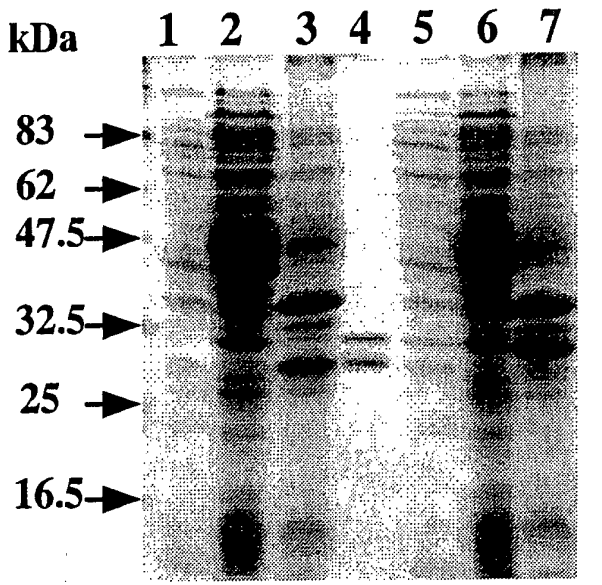


FIG. 3

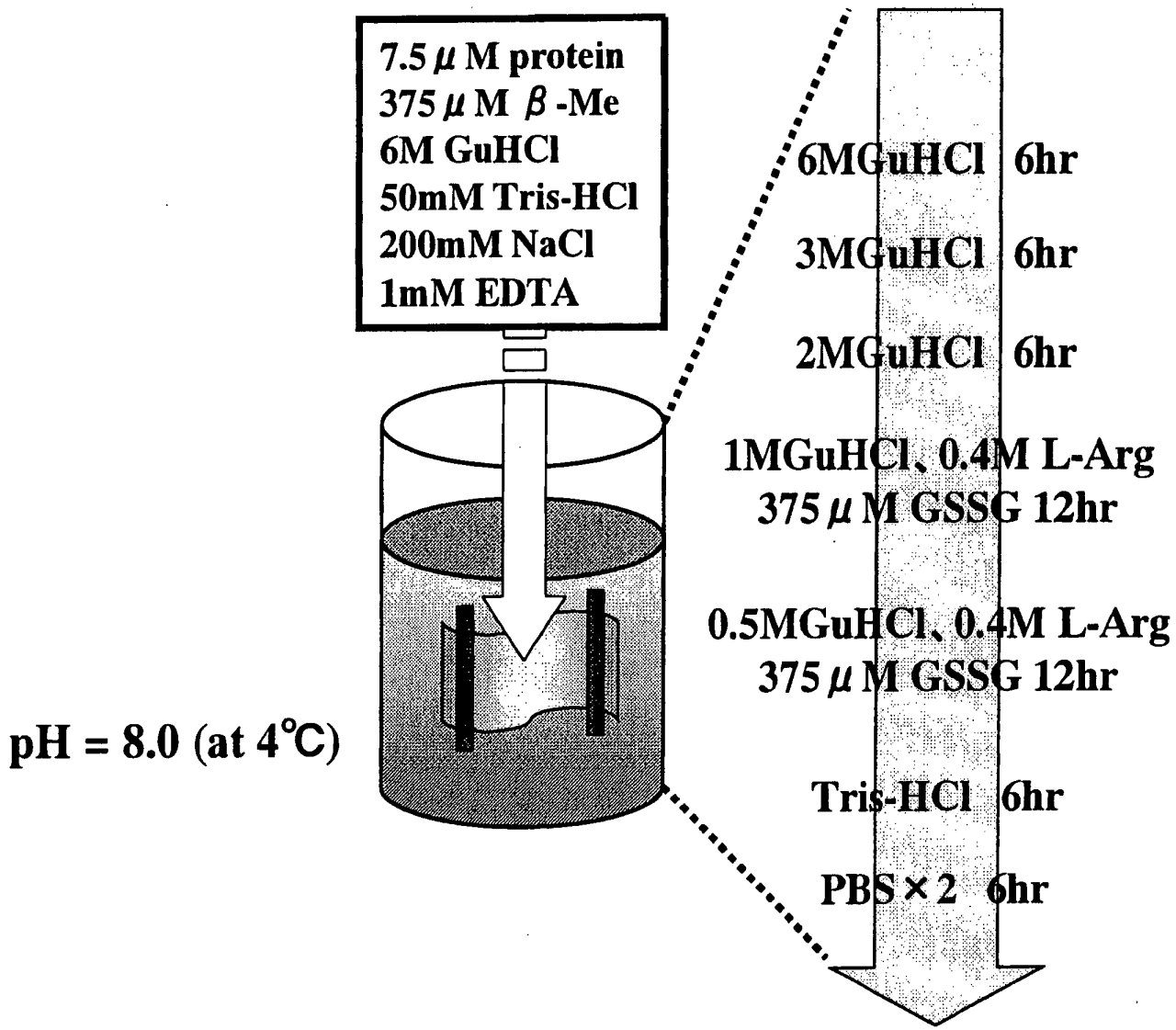
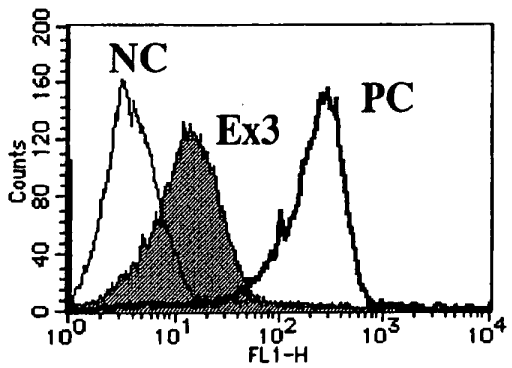
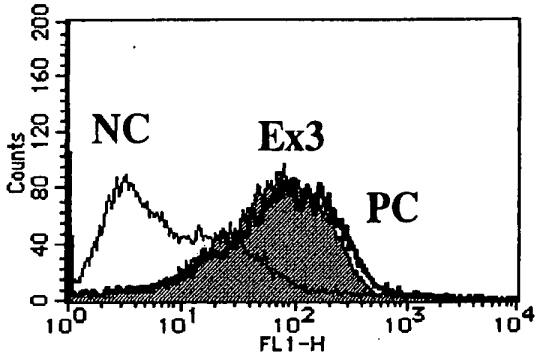

7.5 μ M protein
375 μ M β -Me
6M GuHCl
50mM Tris-HCl
200mM NaCl
1mM EDTA
6MGuHCl 6hr
3MGuHCl 6hr
2MGuHCl 6hr
1MGuHCl、0.4M L-Arg
375 μ M GSSG 12hr
0.5MGuHCl、0.4M L-Arg
375 μ M GSSG 12hr
Tris-HCl 6hr
PBS×2 6hr
pH = 8.0 (at 4℃)

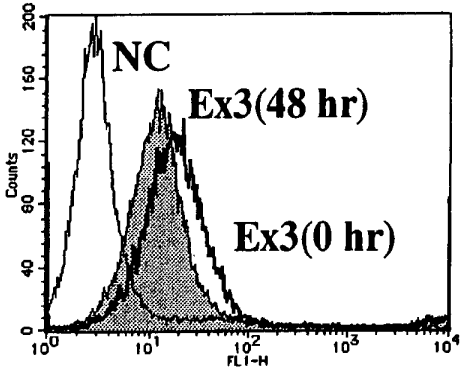
FIG. 4



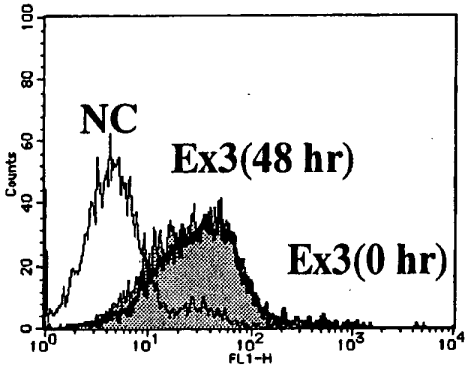
T-LAK (CD3 Positive)



TFK-1 (EGFR Positive)



T-LAK (CD3 Positive)



TFK-1 (EGFR Positive)

FIG. 5

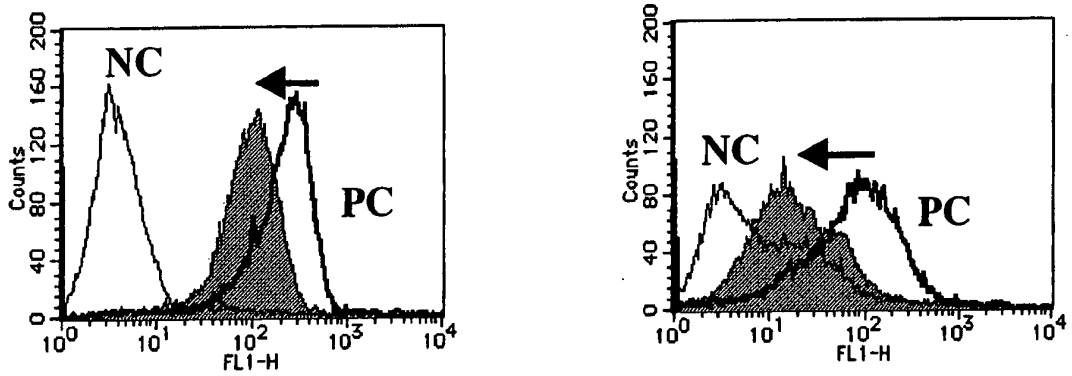

NC
PC
Counts
0
40
80
120
160
200
10^0
10^1
10^2
10^3
10^4
FL1-H
NC
PC
Counts
0
40
80
120
160
200
10^0
10^1
10^2
10^3
10^4
FL1-H

FIG. 6

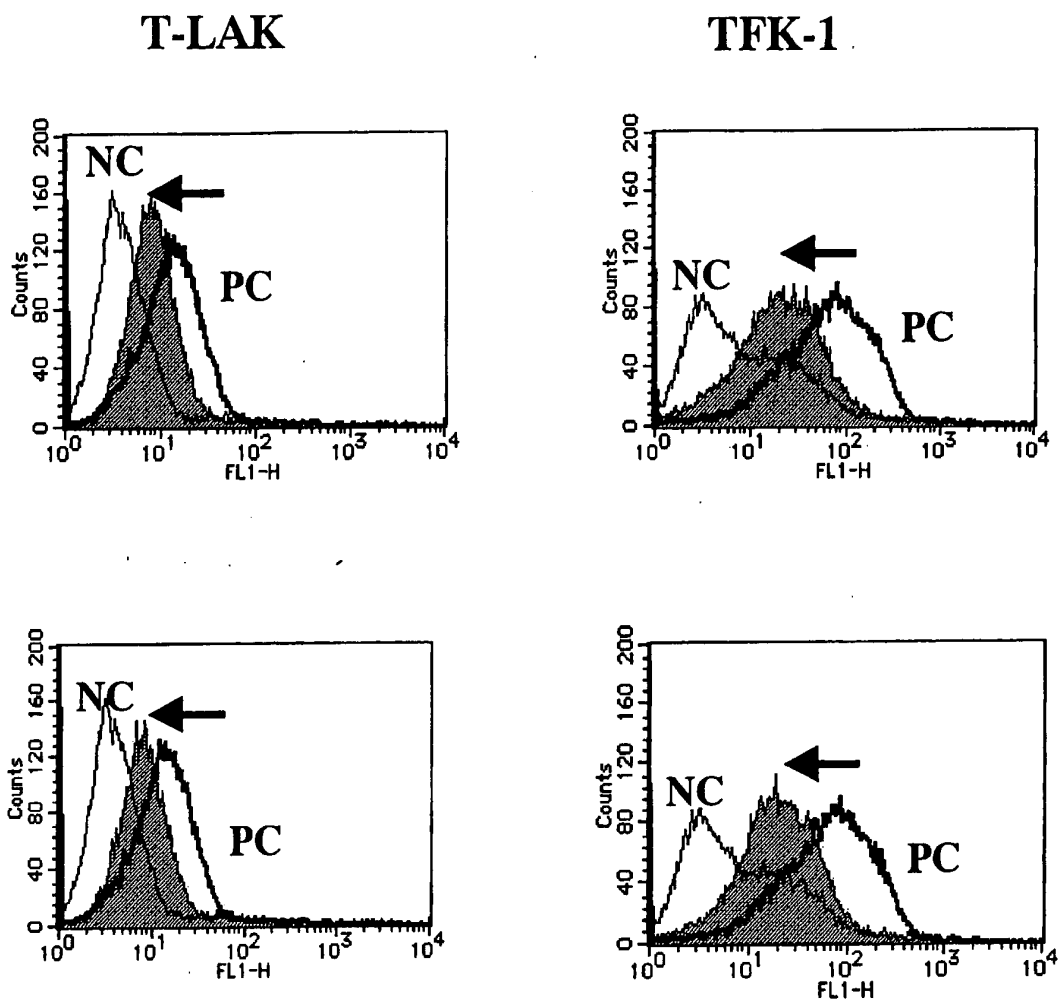


FIG. 7

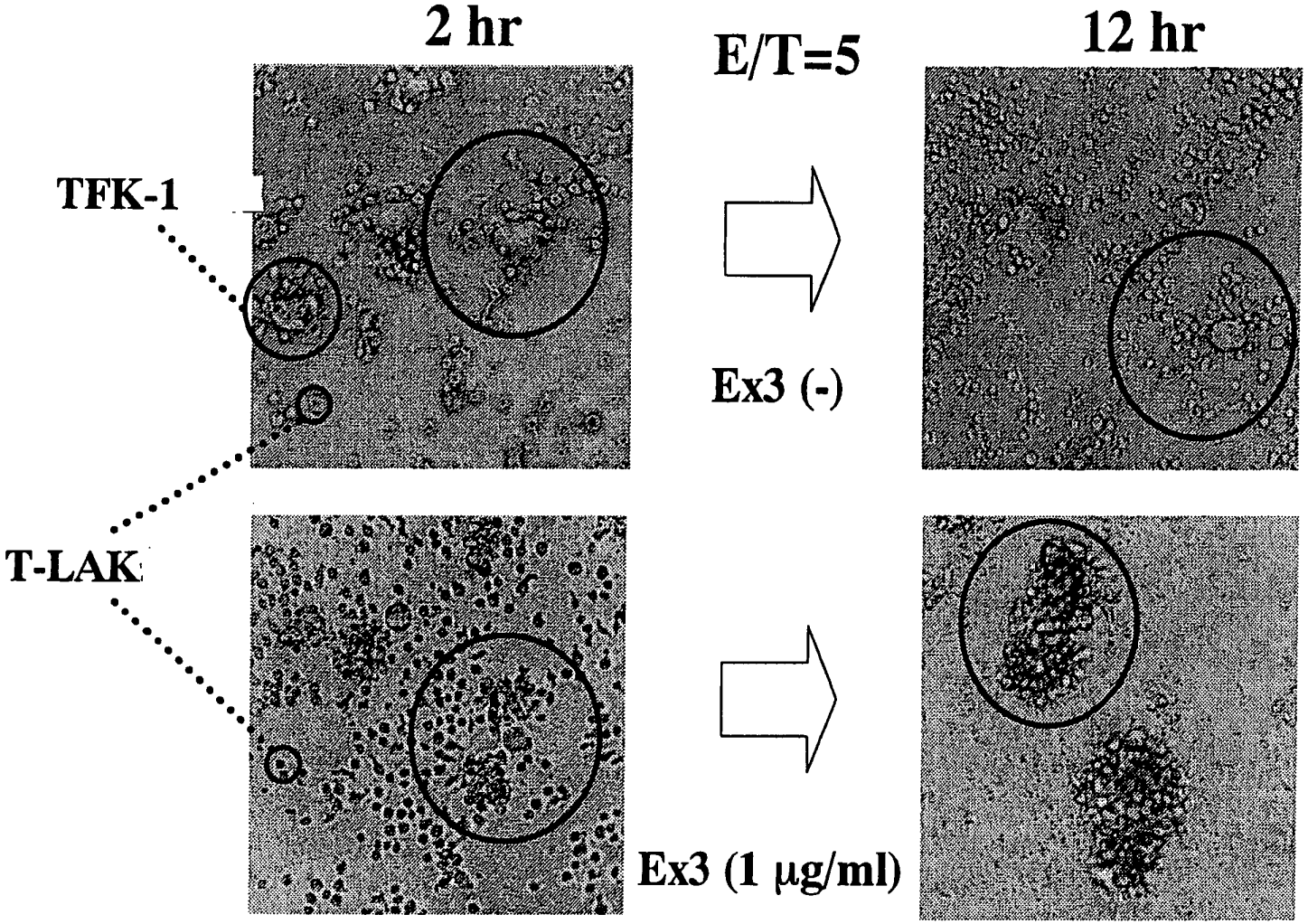
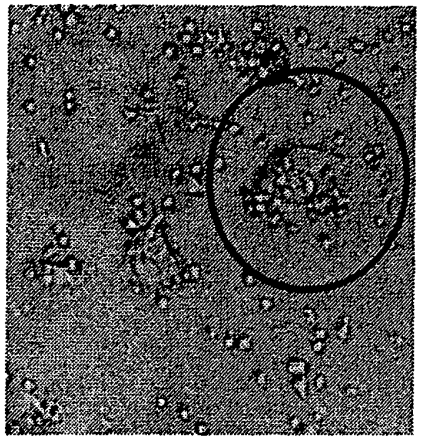


FIG. 8

(E/T=5、18 hr、Ex3 (1 μg/ml))

parental IgG
(1 μg/ml)

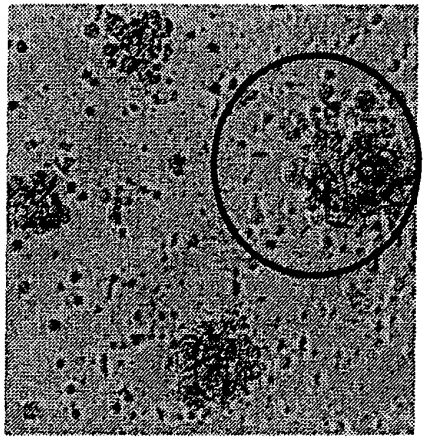


528 (anti-EGFR)

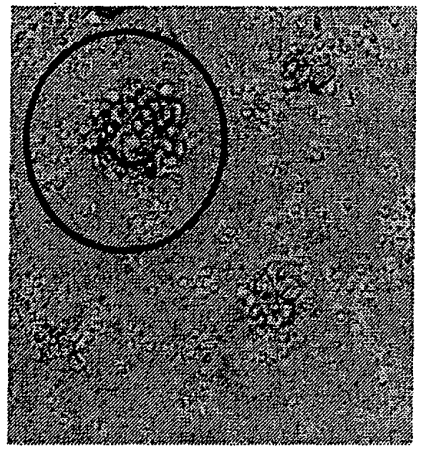
OKT3 (anti-CD3)

inhibited

irrelevant IgG
(1 μg/ml)



MUSE 11 (anti-MUC1)



OKT8 (anti-CD8)

not inhibited

FIG. 9

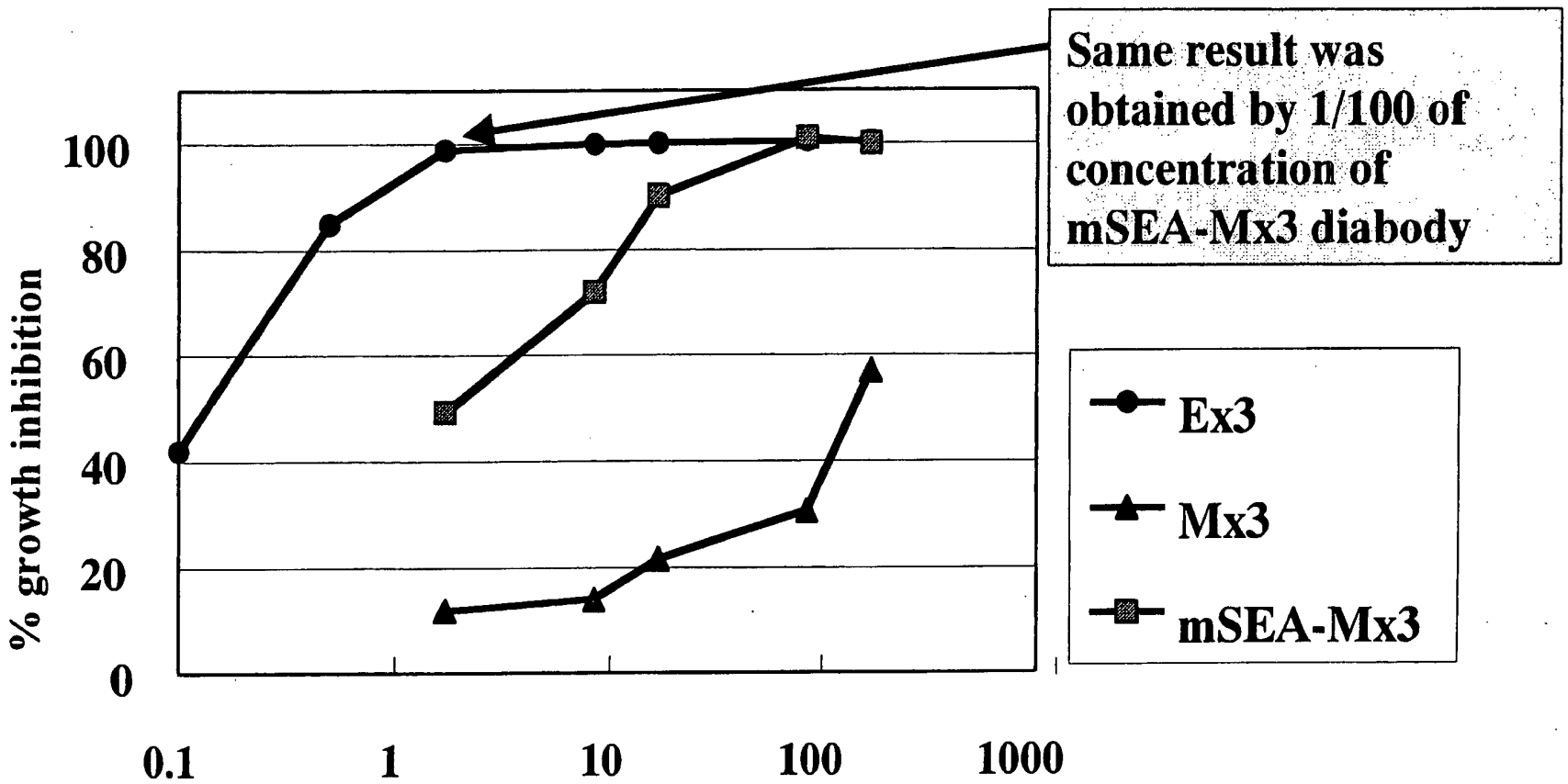

Same result was obtained by 1/100 of concentration of mSEA-Mx3 diabody
% growth inhibition
100
80
60
40
20
0
Ex3
Mx3
mSEA-Mx3
0.1
1
10
100
1000
Log concentration of diabody (pmol/ml)
target cell :TFK-1
(1x10⁴cells/well)
effector cell : T-LAK
Effector/Target=5

FIG. 10

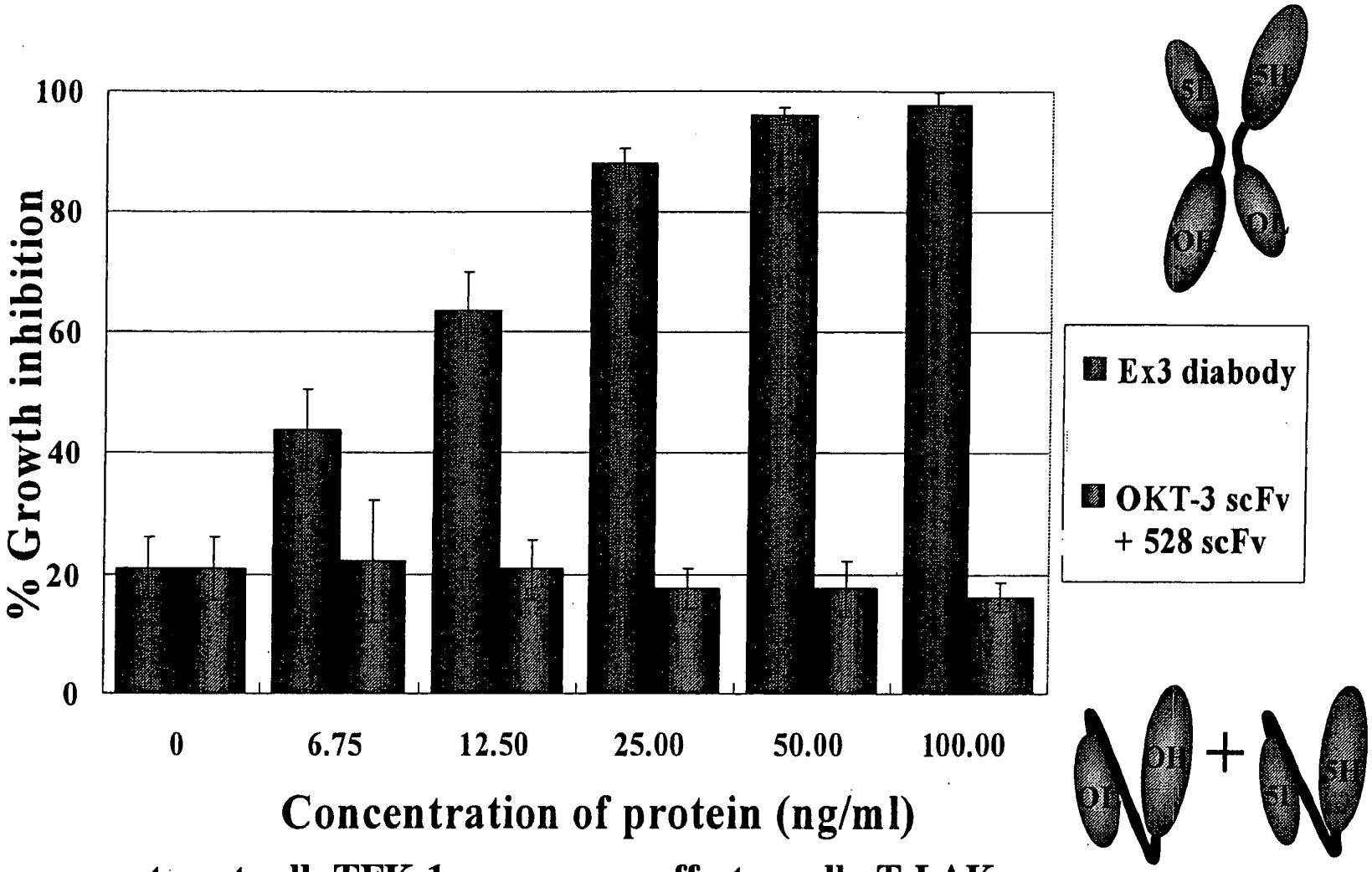

100
80
60
40
20
0
% Growth inhibition
0
6.75
12.50
25.00
50.00
100.00
Concentration of protein (ng/ml)
target cell :TFK-1
(1x10⁴cells/well)
effector cell : T-LAK
Effector/Target=5
Ex3 diabody
OKT-3 scFv
+ 528 scFv

FIG. 11

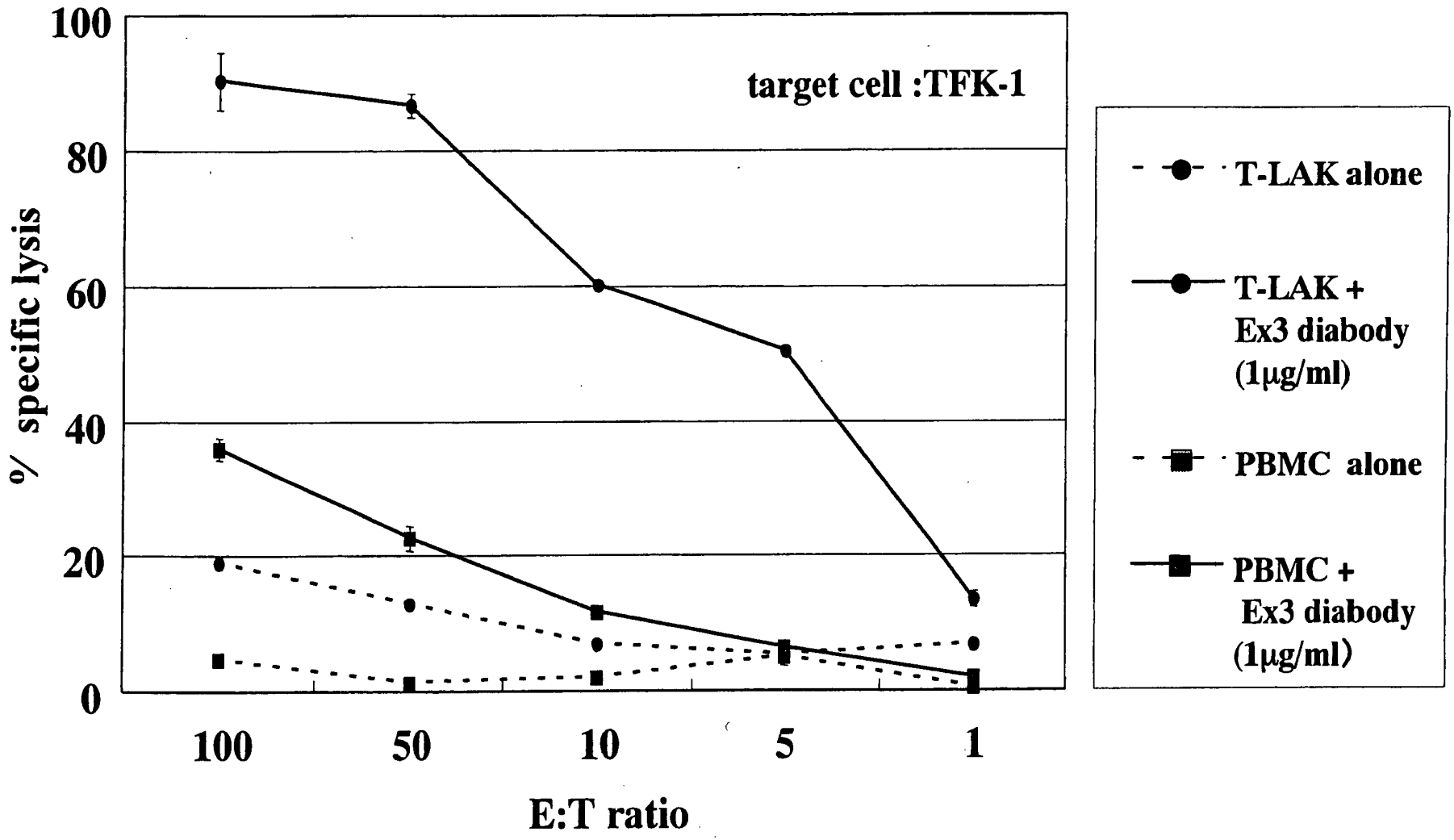

target cell :TFK-1
% specific lysis
100
80
60
40
20
0
100
50
10
5
1
E:T ratio
T-LAK alone
T-LAK + Ex3 diabody (1μg/ml)
PBMC alone
PBMC + Ex3 diabody (1μg/ml)

FIG.12

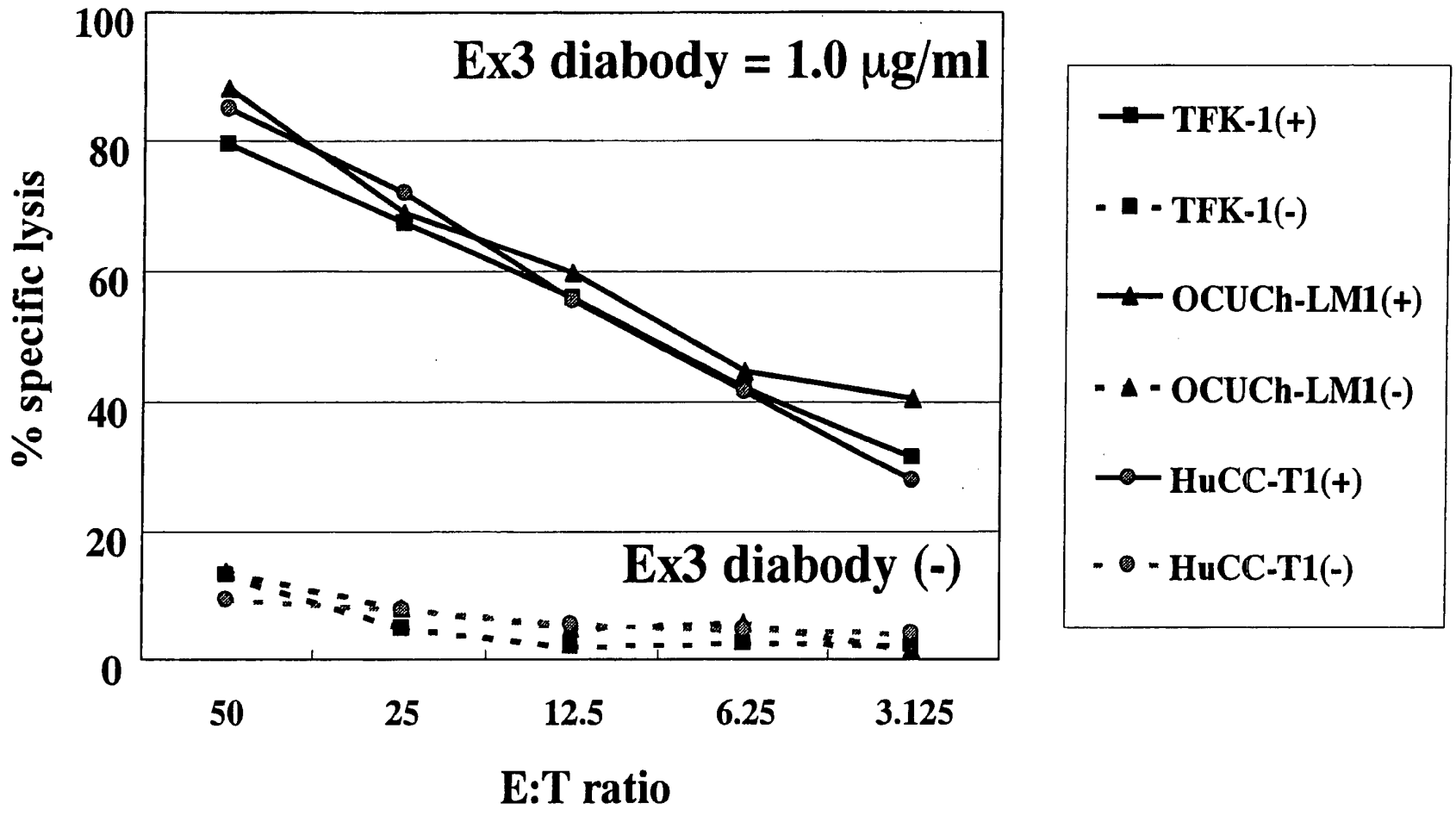

Ex3 diabody = 1.0 μg/ml
Ex3 diabody (-)
% specific lysis
100
80
60
40
20
0
50
25
12.5
6.25
3.125
E:T ratio
TFK-1(+)
TFK-1(-)
OCUCh-LM1(+)
OCUCh-LM1(-)
HuCC-T1(+)
HuCC-T1(-)

FIG. 13

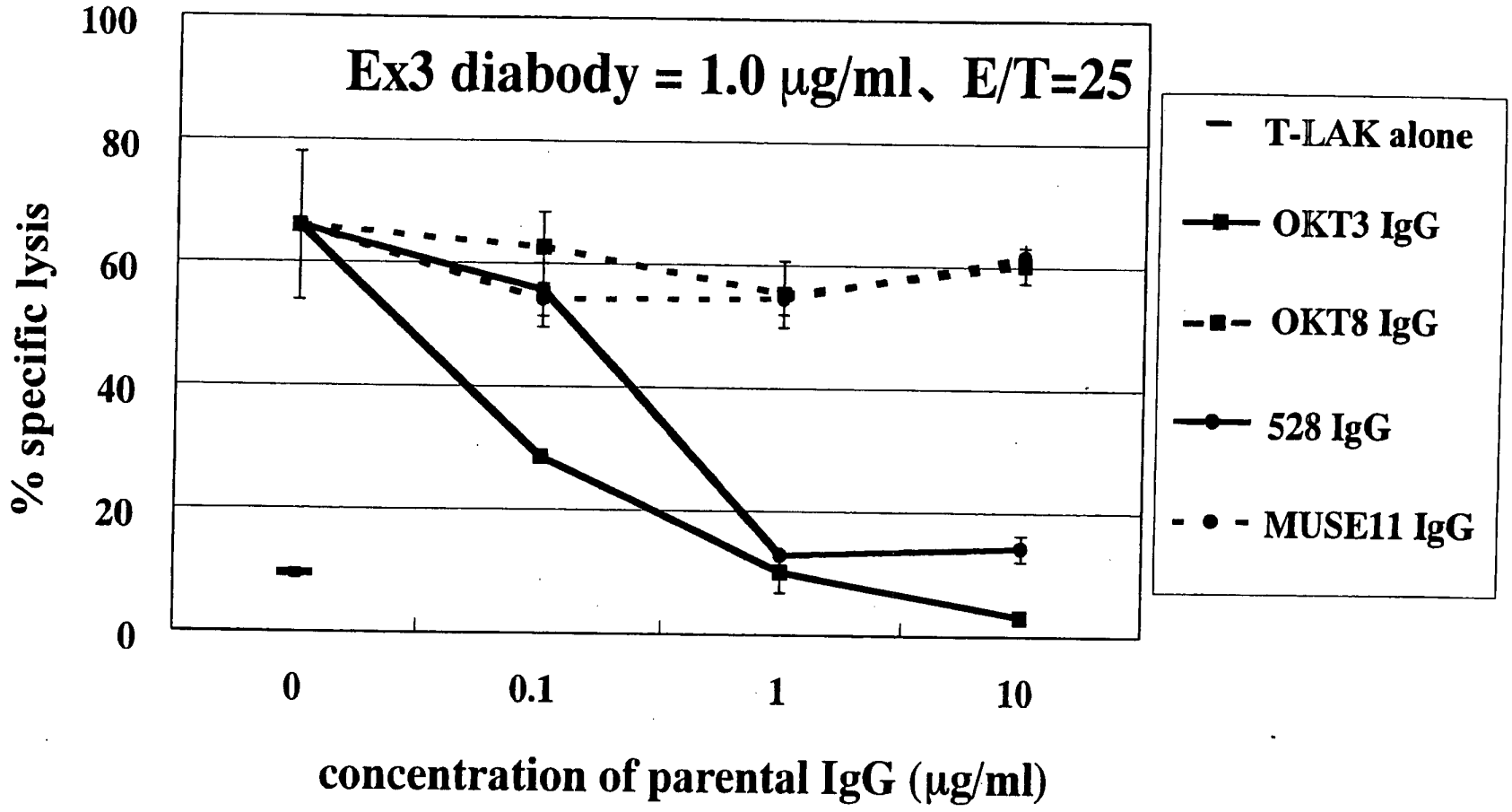
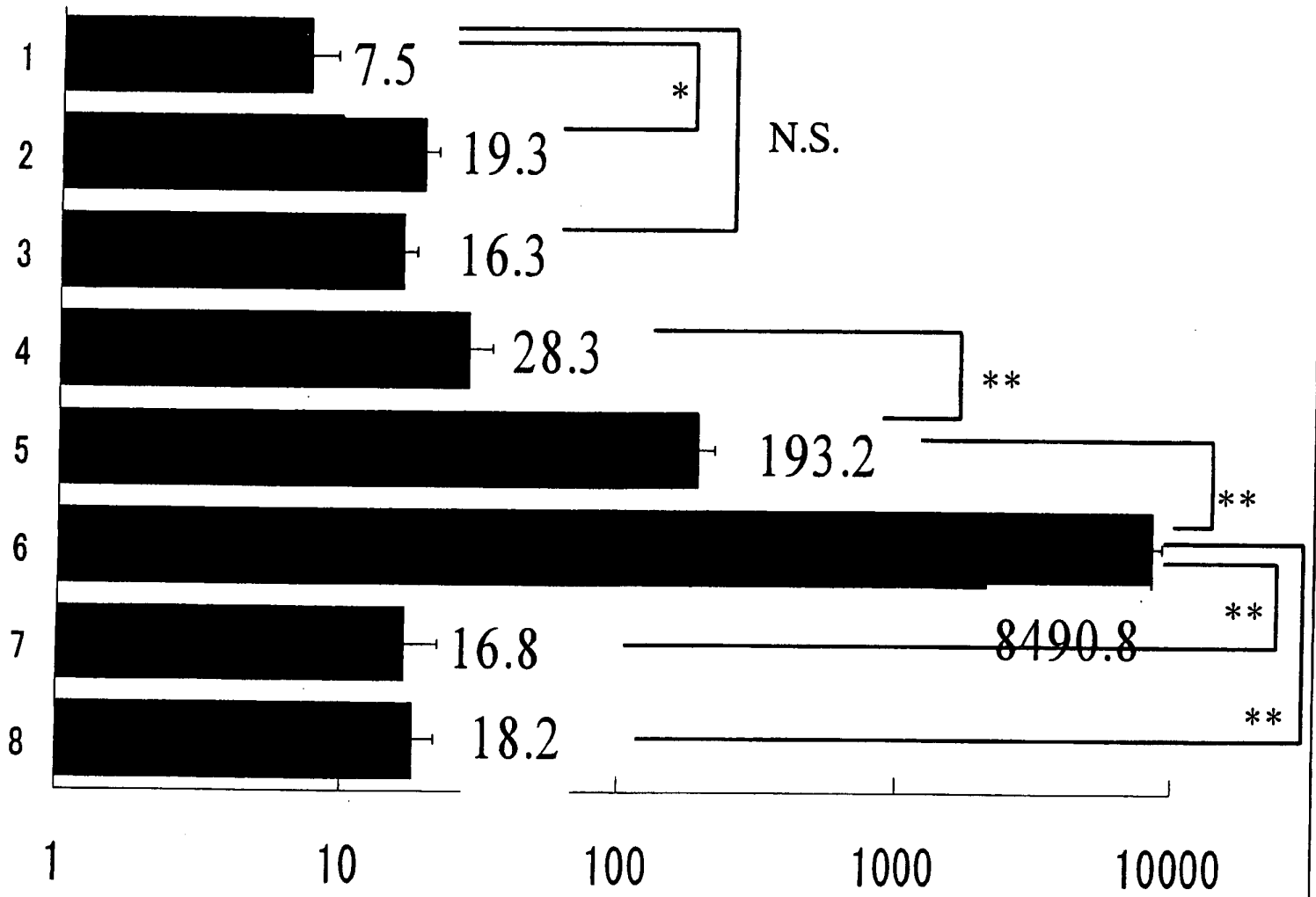
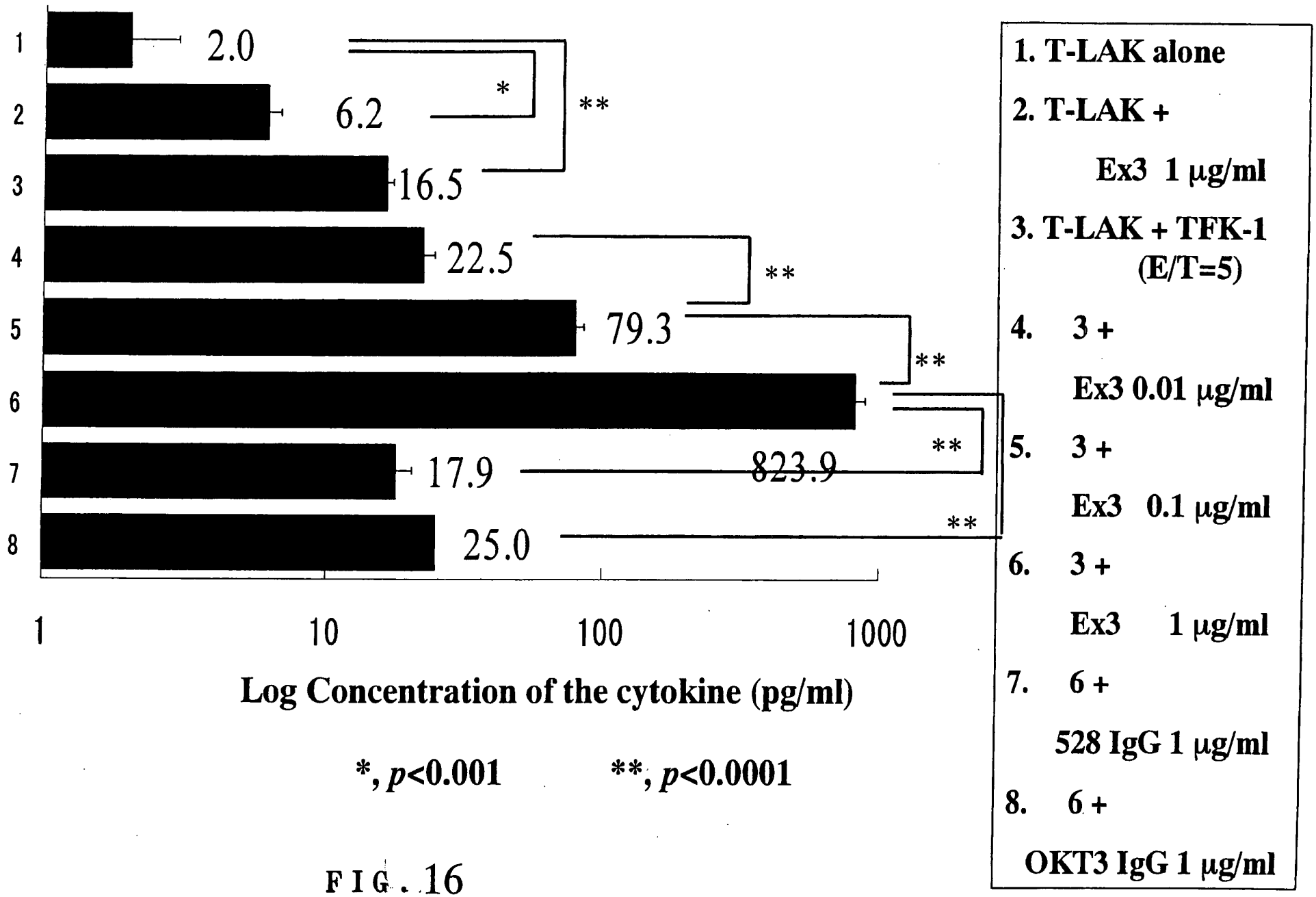

Ex3 diabody = 1.0 μg/ml、E/T=25
% specific lysis
100
80
60
40
20
0
0
0.1
1
10
concentration of parental IgG (μg/ml)
T-LAK alone
OKT3 IgG
OKT8 IgG
528 IgG
MUSE11 IgG

FIG. 14



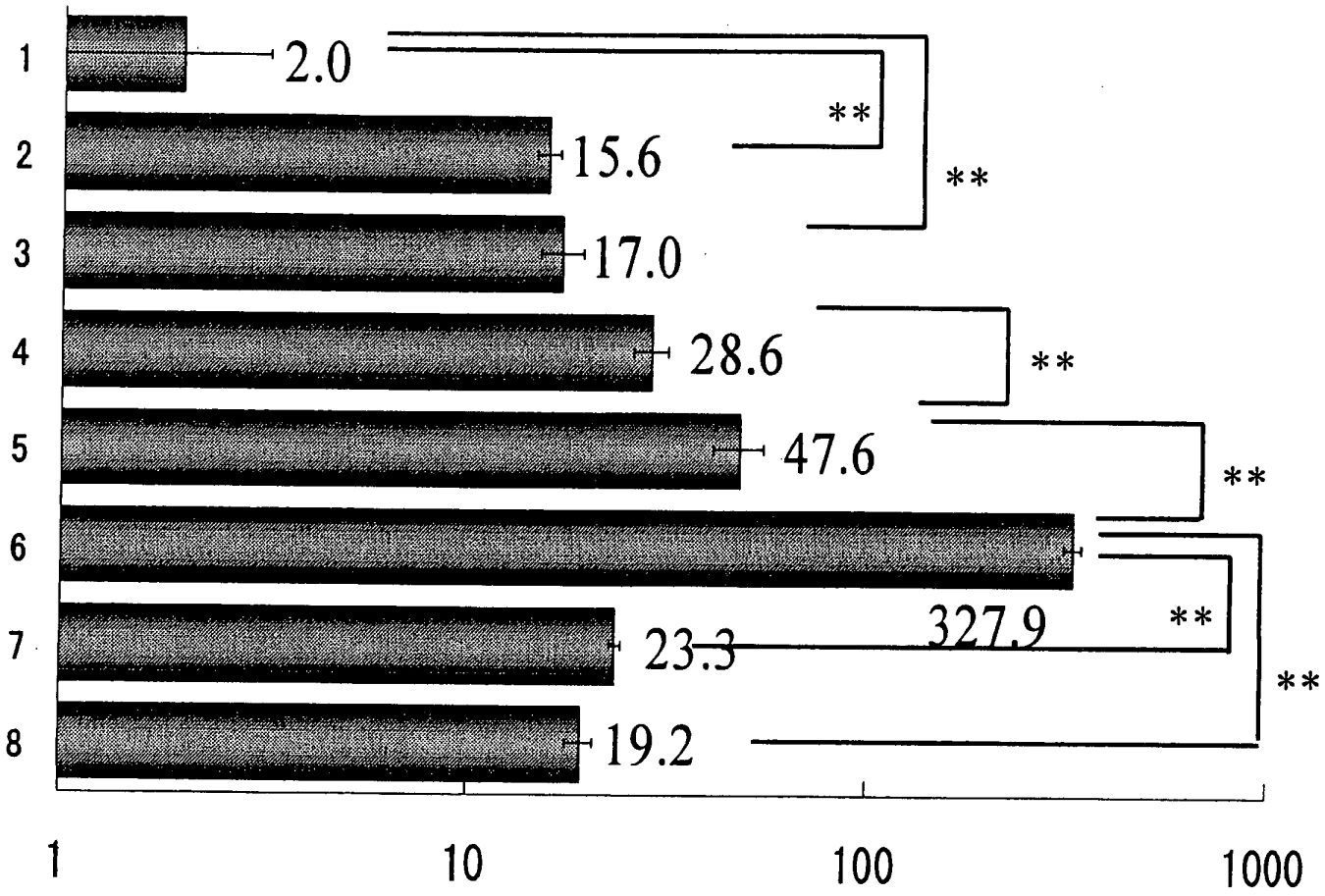
1. T-LAK alone
2. T-LAK + Ex3 1 μg/ml
3. T-LAK + TFK-1 (E/T=5)
4. 3 + Ex3 0.01 μg/ml
5. 3 + Ex3 0.1 μg/ml
6. 3 + Ex3 1 μg/ml
7. 6 + 528 IgG 1 μg/ml
8. 6 + OKT3 IgG 1 μg/ml

FIG. 15



*, $p<0.001$ **, $p<0.0001$

FIG. 16



Log Concentration of the cytokine (pg/ml)

***, $p<0.001$ **, $p<0.0001$**

FIG. 17

1. T-LAK alone

2. T-LAK + Ex3 1 μg/ml

3. T-LAK + TFK-1 (E/T=5)

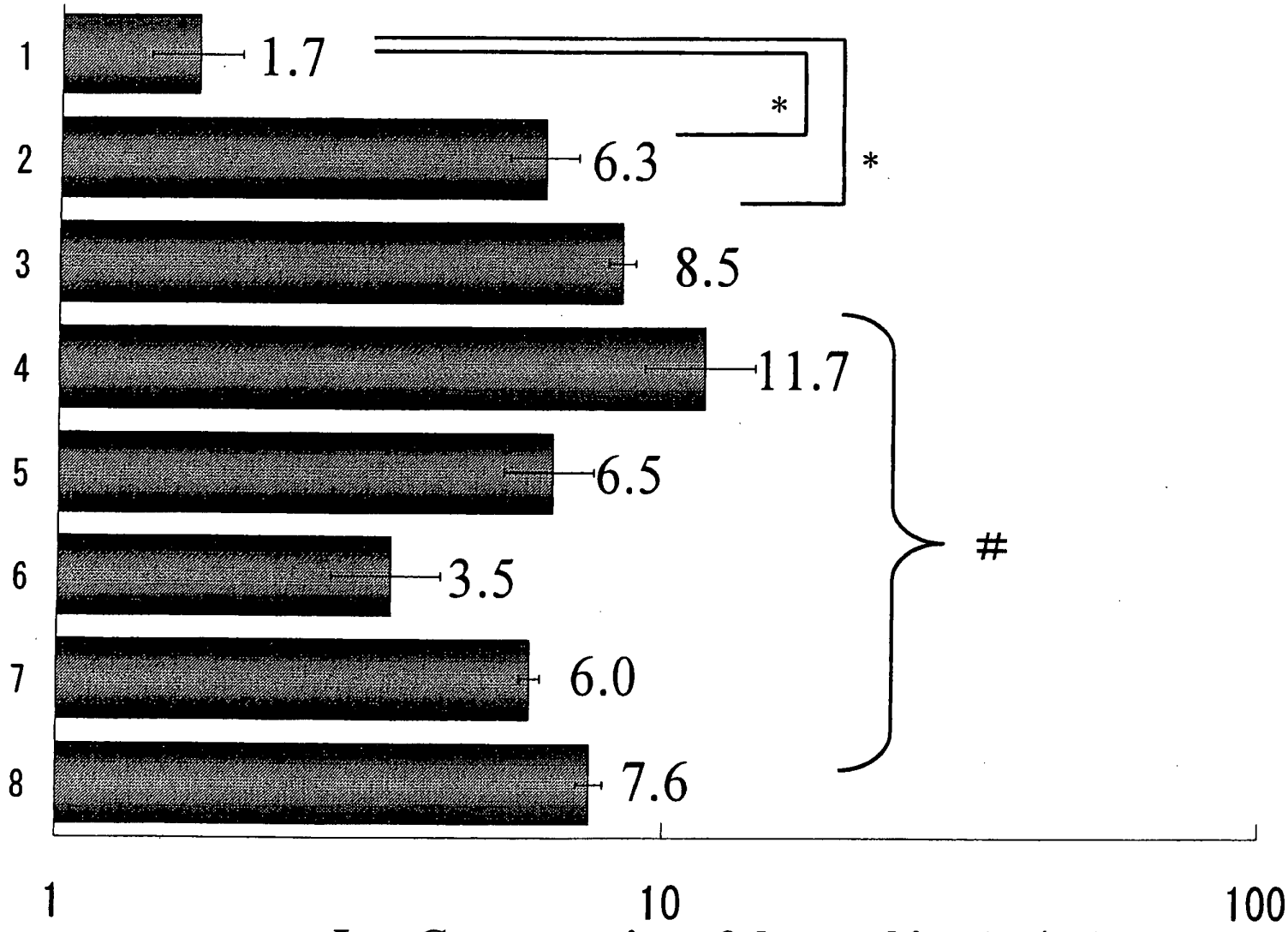
4. 3 + Ex3 0.01 μg/ml

5. 3 + Ex3 0.1 μg/ml

6. 3 + Ex3 1 μg/ml

7. 6 + 528 IgG 1 μg/ml

8. 6 + OKT3 IgG 1 μg/ml



1. T-LAK alone
2. T-LAK + Ex3 1 μg/ml
3. T-LAK + TFK-1 (E/T=5)
4. 3 + Ex3 0.01 μg/ml
5. 3 + Ex3 0.1 μg/ml
6. 3 + Ex3 1 μg/ml
7. 6 + 528 IgG 1 μg/ml
8. 6 + OKT3 IgG 1 μg/ml

*, $p<0.001$ #, There was no significant difference between every 2 groups.

FIG. 18

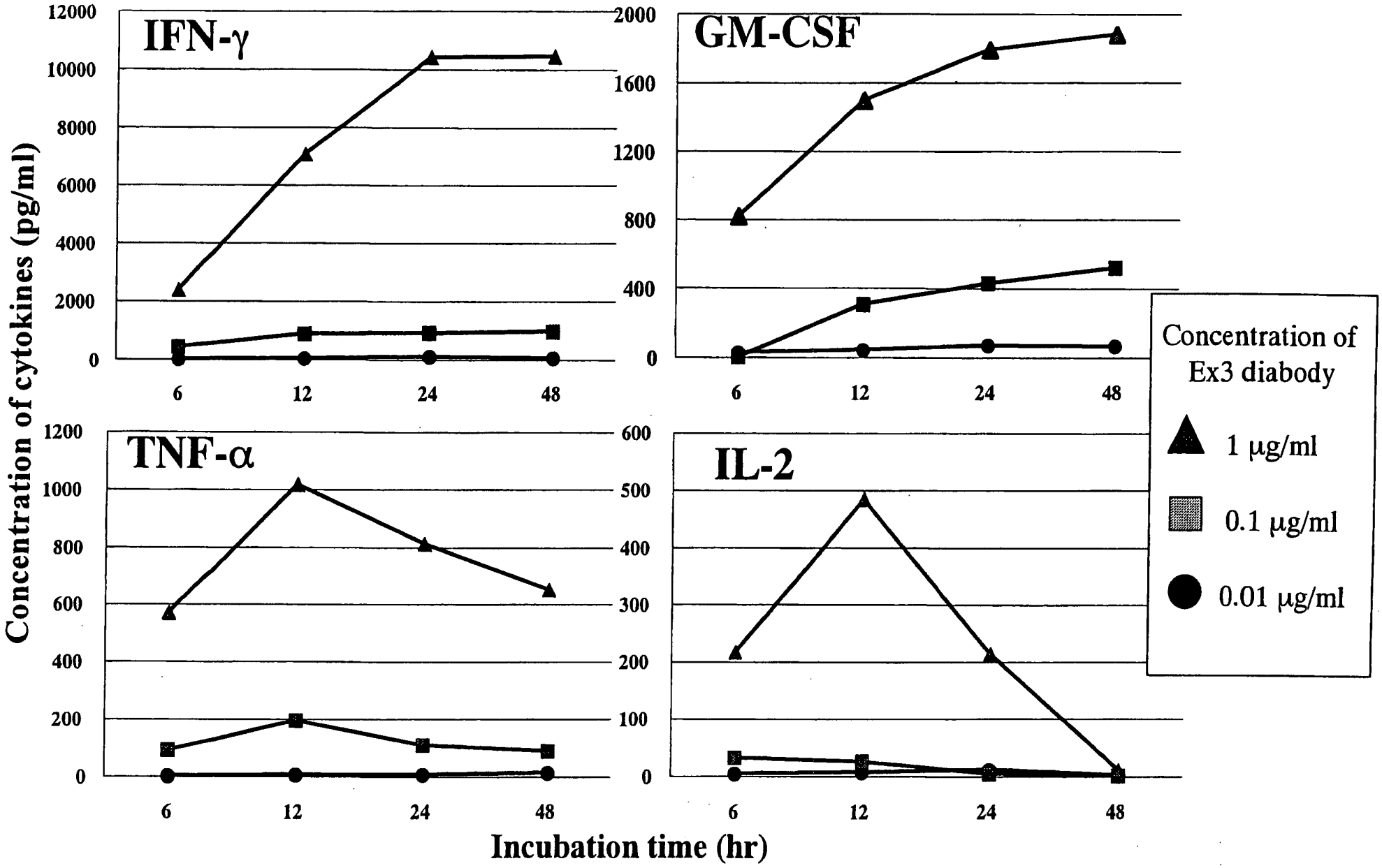

IFN-γ
GM-CSF
TNF-α
IL-2
Concentration of cytokines (pg/ml)
Incubation time (hr)
Concentration of Ex3 diabody
1 μg/ml
0.1 μg/ml
0.01 μg/ml
12000
10000
8000
6000
4000
2000
0
2000
1600
1200
800
400
0
1200
1000
800
600
400
200
0
600
500
400
300
200
100
0
6
12
24
48

FIG. 19

SCID mouse

$5x10^6$ TFK-1 s.c.

10 days after (about 5 mm in diameter)

2×10^7 T-LAK(IL-2; 500IU) with diabody i.v. (for 4 consecutive days)

tumor volume is calculated as $(\text{width})^2 \times \text{length} / 2$

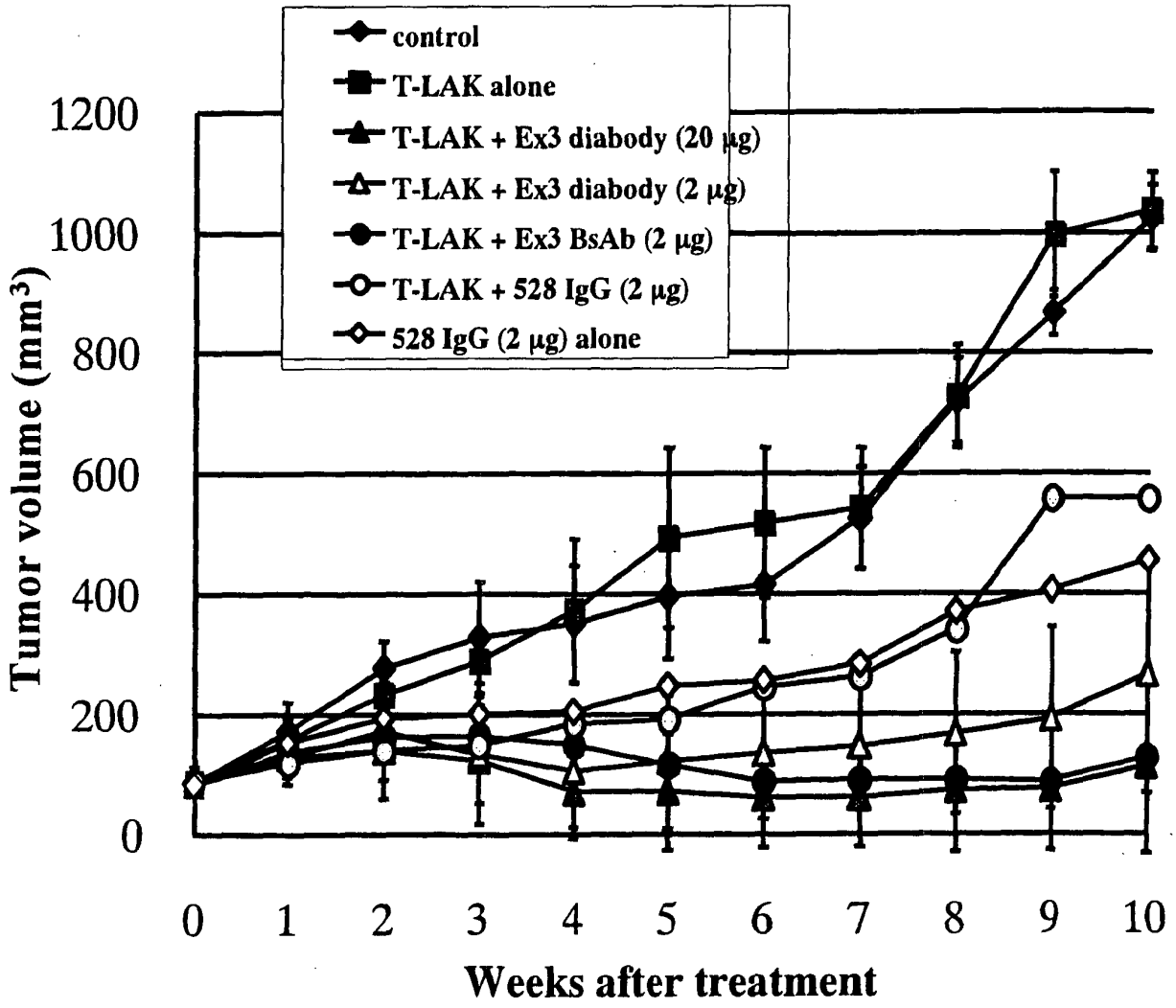


FIG. 20

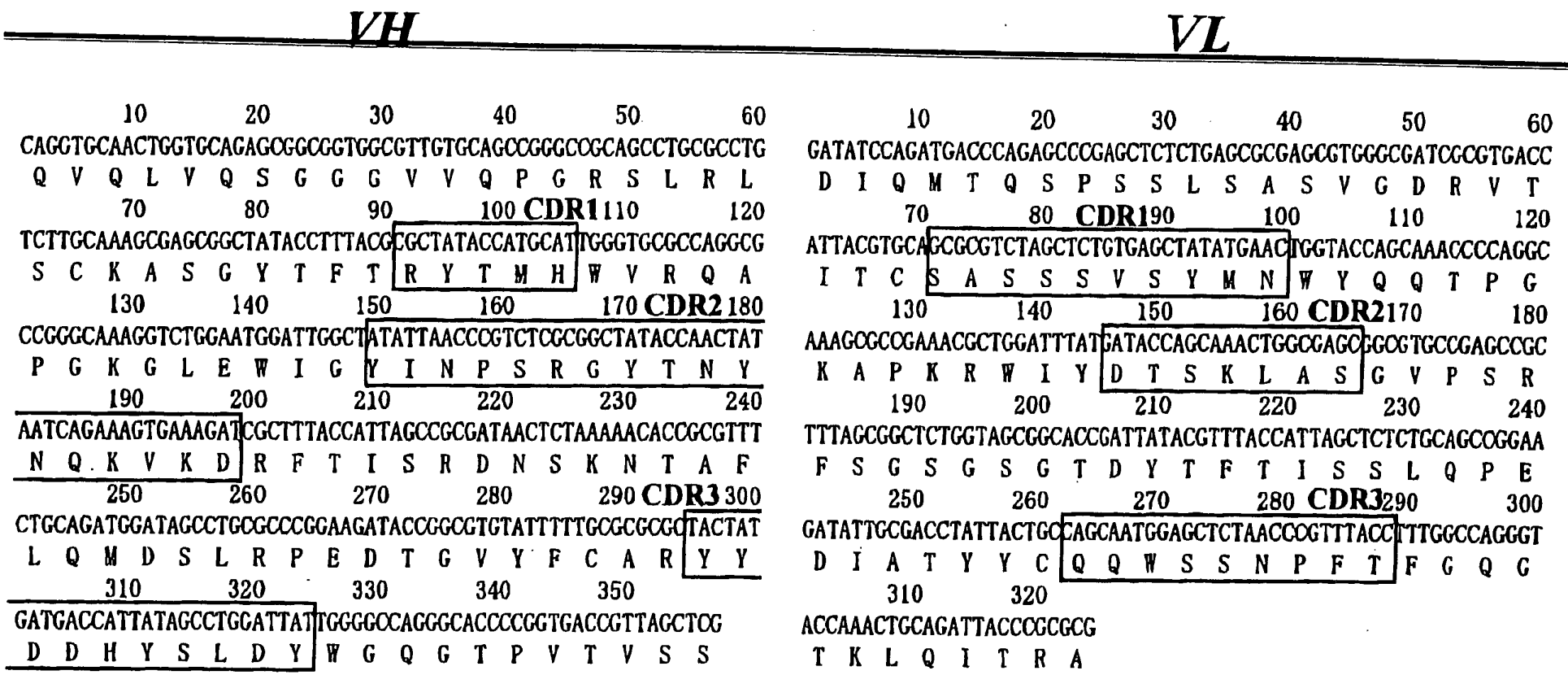


FIG. 21

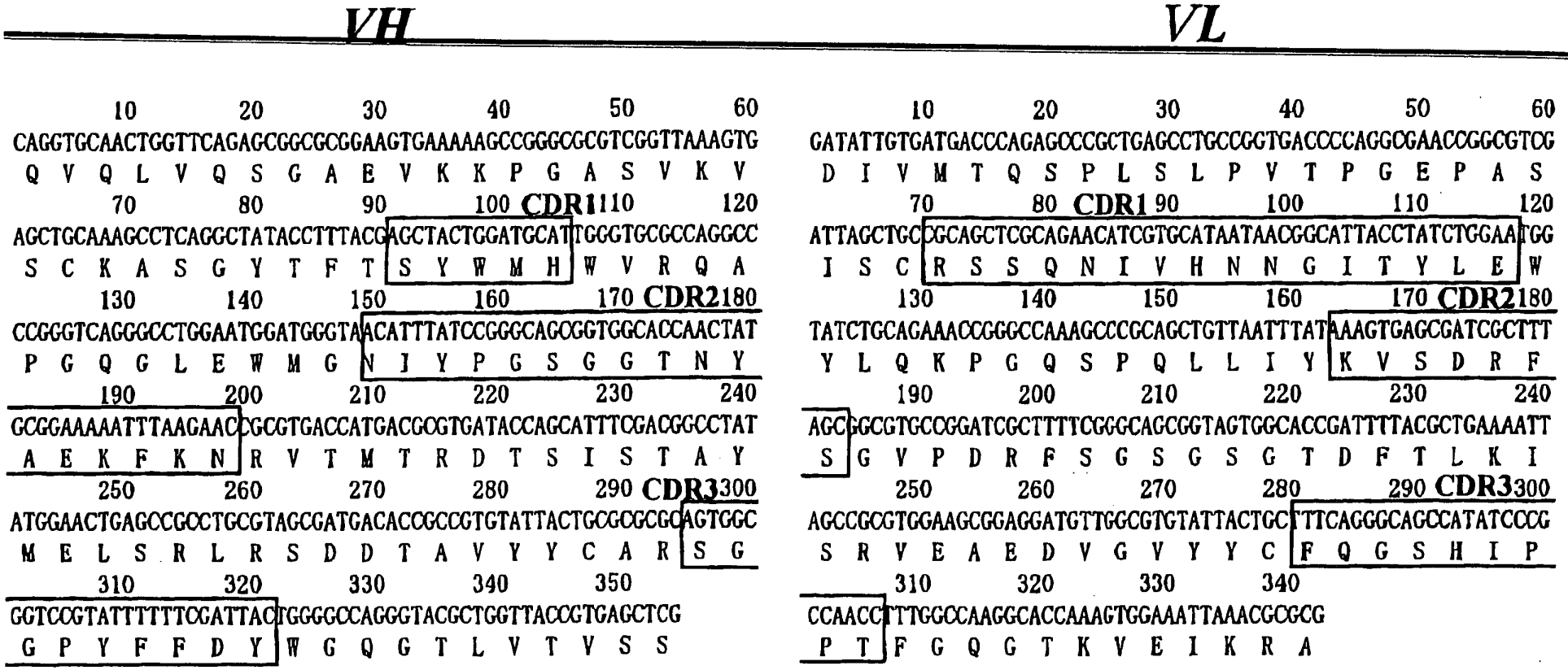


FIG. 22

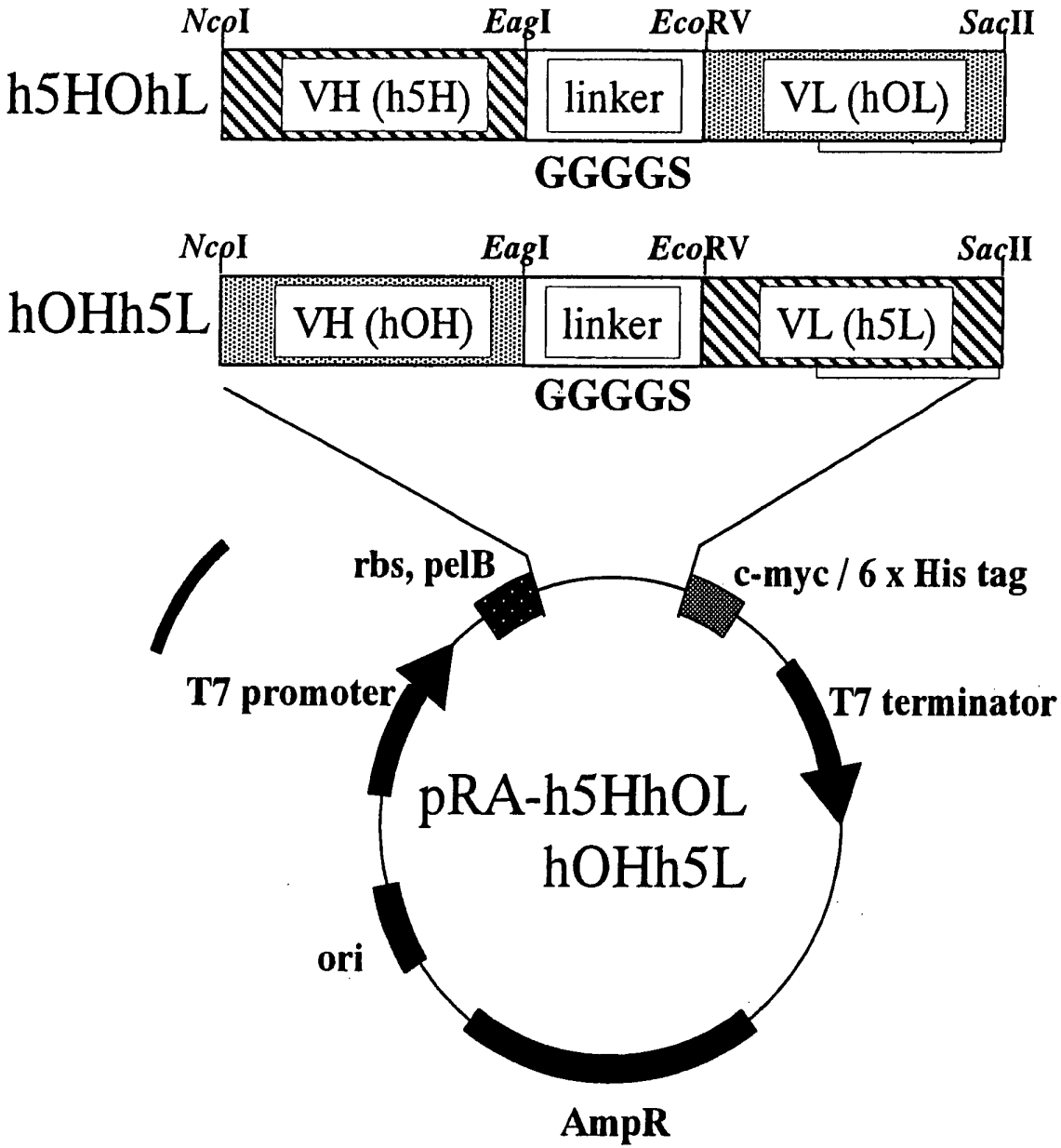
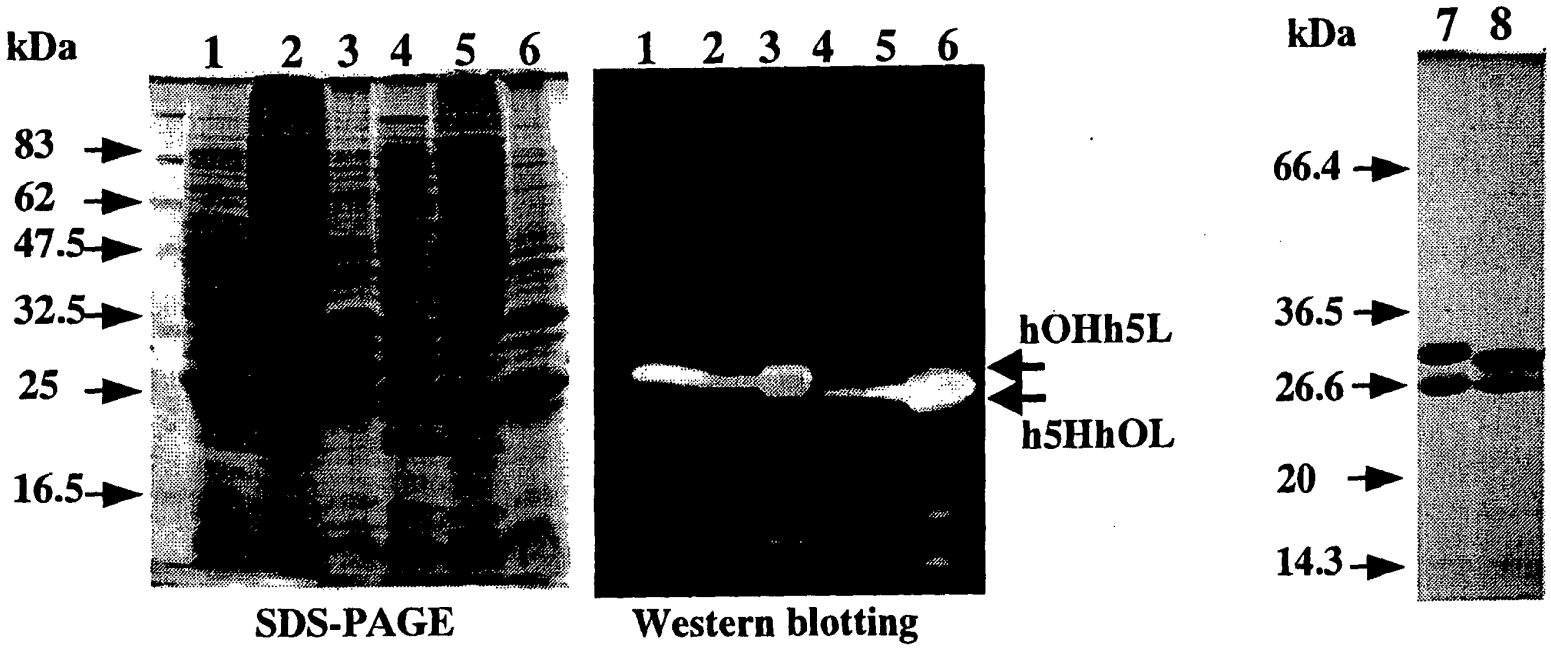

NcoI
EagI
EcoRV
SacII
h5HOhL
VH (h5H)
linker
VL (hOL)
GGGGS
NcoI
EagI
EcoRV
SacII
hOHh5L
VH (hOH)
linker
VL (h5L)
GGGGS
rbs, pelB
c-myc / 6 x His tag
T7 promoter
T7 terminator
pRA-h5HhOL
hOHh5L
ori
AmpR

FIG. 23


kDa
1 2 3 4 5 6
83
62
47.5
32.5
25
16.5
SDS-PAGE
1 2 3 4 5 6
hOHh5L
h5HhOL
Western blotting
kDa
7 8
66.4
36.5
26.6
20
14.3

FIG, 24

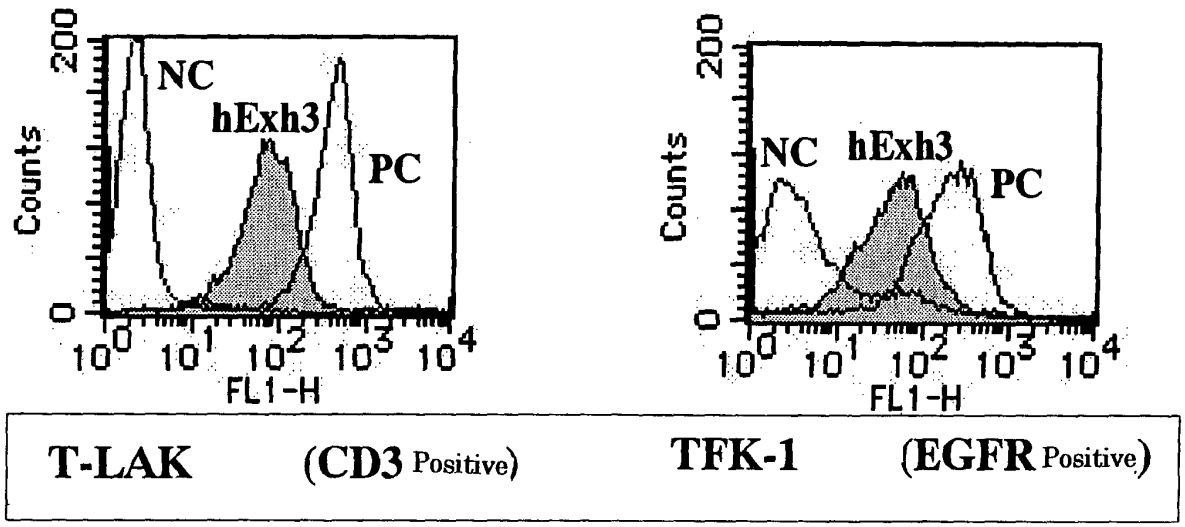

200
0
Counts
NC
hExh3
PC
10^0 10^1 10^2 10^3 10^4
FL1-H
T-LAK (CD3 Positive)
200
0
Counts
NC
hExh3
PC
10^0 10^1 10^2 10^3 10^4
FL1-H
TFK-1 (EGFR Positive)

FIG. 25

	FR1	CDR1	FR2	CDR2
5H	QVQLQQSGSEMARPGASVKLPCKASGDTFT	SYWMH	WVKQRHGHGPEWIG	NIYPGSGGTNYAEKFKN
h5H	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	SYWMH	WVRQAPGQGLEWMG	NIYPGSGGTNYAEKFKN
h5H-m01	----------	-----	----------I-	----------
h5H-m02	----------	-----	----------I-	----------
h5H-m03	----------	-----	----------	----------
h5H-m04	----------	-----	----------I-	----------
h5H-m05	----------D---	-----	----------I-	----------
h5H-m06	----------	-----	----------I-	----------
h5H-m07	----------	-----	----------	----------
h5H-m08	----------	-----	----------I-	----------
h5H-m09	----------	-----	----------I-	----------
h5H-m10	----------	-----	----------I-	----------

	FR3	CDR3	FR4
5H	KVTLTVDRSSRTVYMHLSRLTSEDSAVYYCTR	SGGPYFFDY	WGQGTTLTVSS
h5H	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR	SGGPYFFDY	WGQGTLVTVSS
h5H-m01	----------	---------	----------
h5H-m02	----------T-	---------	----------
h5H-m03	K----V----------	---------	----------
h5H-m04	K----V----------	---------	----------
h5H-m05	K----V----------	---------	----------
h5H-m06	K----V----------T-	---------	----------
h5H-m07	K--L-V-R----------	---------	----------
h5H-m08	K--L-V-R----------	---------	----------
h5H-m09	K--L-V-R----------T-	---------	----------
h5H-m10	K----V---SR-V----------T-	---------	----------

FIG. 26

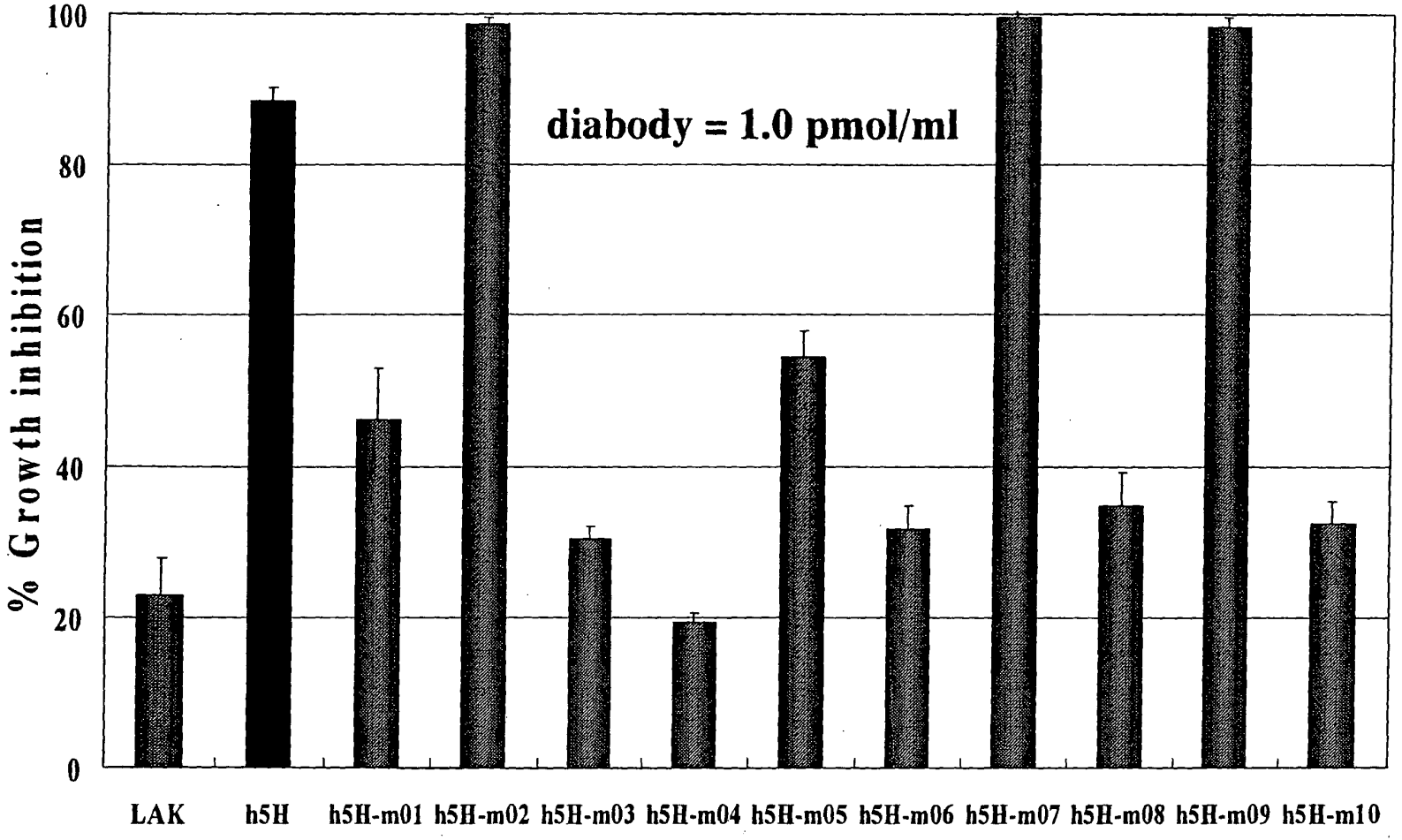

diabody = 1.0 pmol/ml
% Growth inhibition
100
80
60
40
20
0
LAK
h5H
h5H-m01
h5H-m02
h5H-m03
h5H-m04
h5H-m05
h5H-m06
h5H-m07
h5H-m08
h5H-m09
h5H-m10

FIG. 27

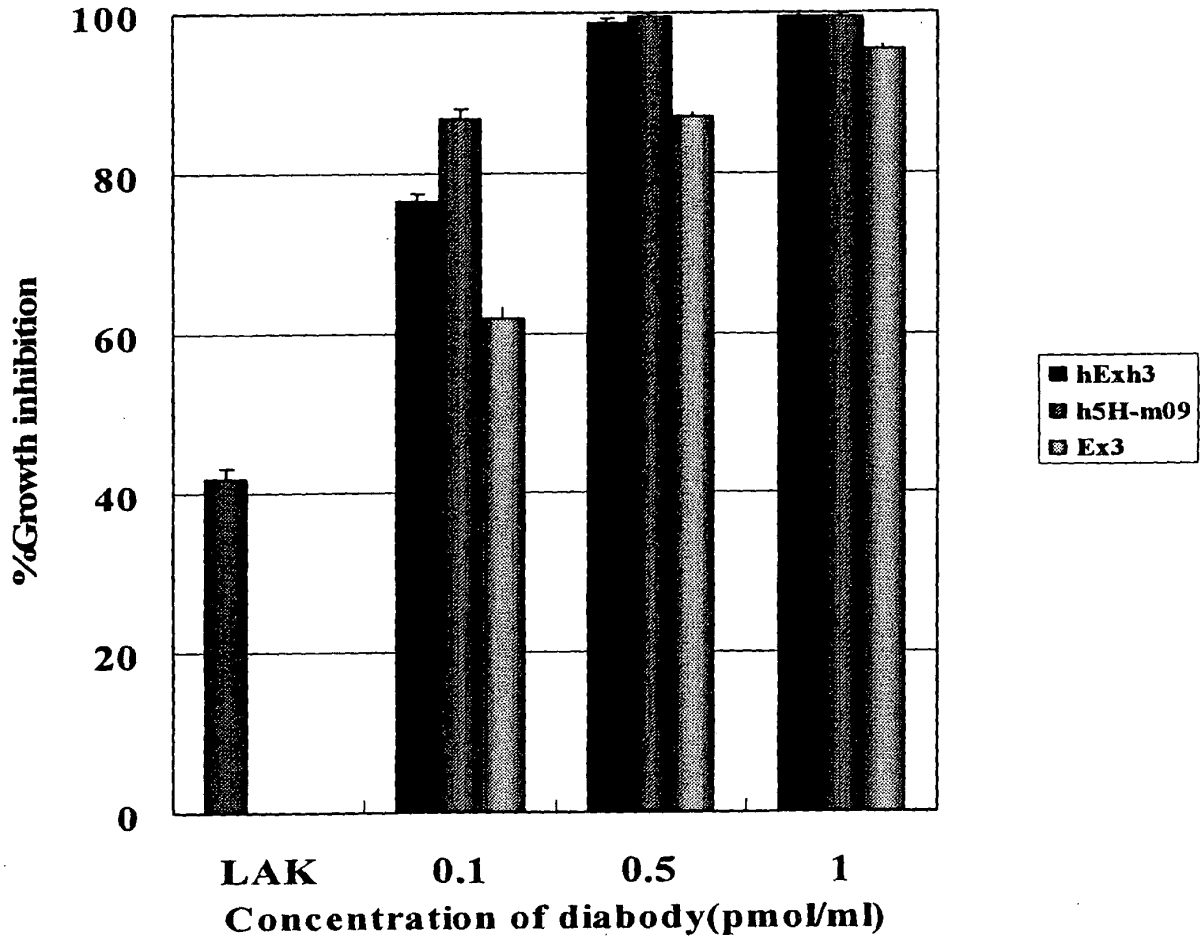

100
80
60
40
20
0
%Growth inhibition
LAK
0.1
0.5
1
Concentration of diabody(pmol/ml)
hExh3
h5H-m09
Ex3

FIG. 28